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Uncovering Nature's Design Strategies through Parametric Modeling, Multi-Material 3D Printing, and Mechanical Testing**

By Simon Frølich, James C. Weaver,* Mason N. Dean and Henrik Birkedal*

Nature produces a multitude of composite materials with intricate architectures that in many instances far exceed the performance of their modern engineering analogs. Despite significant investigations into structure-function relationships of complex biological materials, there is typically a lack of critical information regarding the specific functional roles of many of their components. To help resolve this issue, the authors present here a framework for investigating biological design principles that combines parametric modeling, multi-material 3D printing, and direct mechanical testing to efficiently examine very large parameter spaces of biological design. Using the brick and mortar-like architecture of mollusk nacre as a model system, the authors show that this approach can be used to effectively examine the structural complexity of biological materials and harvest design principles not previously accessible.

Biological systems continue to serve as sources of inspiration for innovative materials design as they often feature surprising combinations of properties owing to their hierarchical and multiphase architectures.^[1] While many attempts have been made at adapting these biological design strategies to solving real world engineering problems,^[1b,2] the fact that biology is characterized by multi-purpose systems that interact in complex ways, makes it challenging to tease

apart specific cause and effect relationships in biological materials design. If we are to succeed in mimicking biology in order to achieve a specific performance metric, it is necessary to establish relationships that identify the design parameters that are essential to materials' mechanical performance and not simply artifacts of growth processes or evolutionary baggage (i.e., features maintained in current populations but no longer advantageous to species' fitness). Our limitations in translating biological designs are in very large part due to the lack of efficient and high resolution methods for querying the 3D "structural parameter space" of biological systems – the range of observed and/or possible biological morphologies – to allow distillation of the most decisive features.

To this end, we have developed a new framework for the study of biological materials design, combining parametric modeling, multi-material 3D printing, and direct mechanical testing (Figure 1), thereby adding to previously developed methodologies.^[3] Specifically, this modified framework allows for investigations of 3D biological structures in a systematic and parametric manner, whereas previous methods have focused on the correlation between 2D computational models and the mechanical behavior of their 3D-printed analogs (i.e. 2D models extruded into 3D).^[3a-c,e] In addition, our framework relies on rich feedback loops among techniques that have their roots in architecture, biology, materials science, engineering, and manufacturing, resulting in synergistic perspectives on biological materials that enrich all of these fields. The first step involves identification of the structural features and material

[*] Assoc. Prof. Dr. H. Birkedal, Dr. S. Frølich
iNANO and Department of Chemistry, Aarhus University, 14
Gustav Wieds Vej, 8000 Aarhus, Denmark
hbirkedal@chem.au.dk

Dr. J. C. Weaver
Wyss Institute for Biologically Inspired Engineering, Harvard
University, 60 Oxford St. Cambridge, MA 02138, USA
E-mail: james.weaver@wyss.harvard.edu

Dr. M. N. Dean
Max Planck Institute of Colloids and Interfaces, Department of
Biomaterials, Am Muehlenberg 1, 14424 Potsdam, Germany

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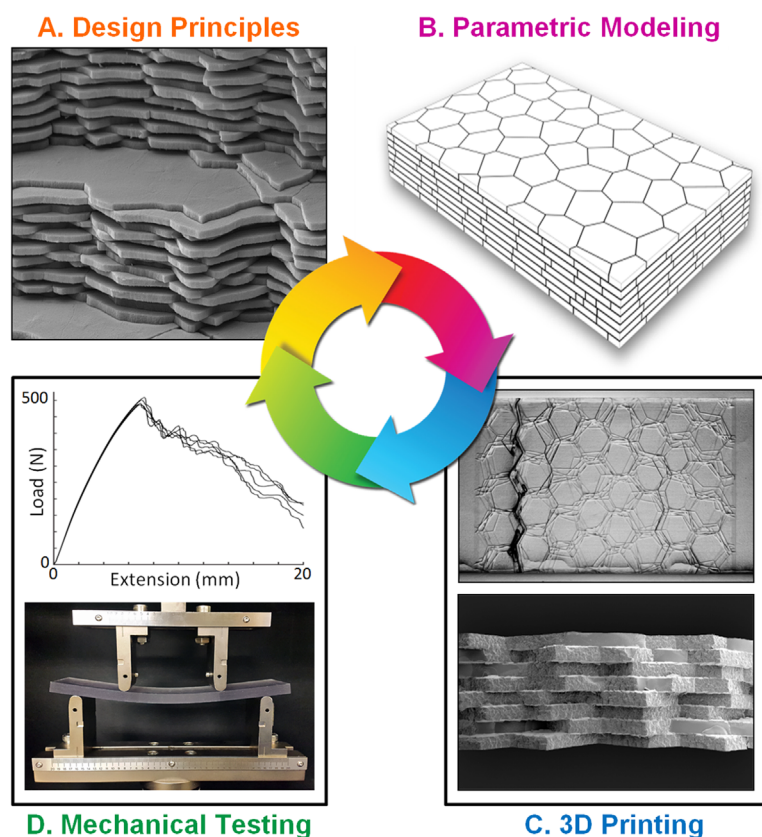


Fig. 1. The cyclic framework used to extract design principles informing both biological and engineering knowledge. (a) Initial structural information is harvested from investigations of biological materials (SEM image of nacre with tablet thickness of ca. 300 nm). (b) The design principles are translated into a fully parametric model encompassing all relevant features (3D rendering of a specific nacre model). (c) Multi-material 3D printing is used to rapidly fabricate samples. The upper panel is a photograph of one of the printed models post-failure and the lower panel is an SEM image of the resulting fracture surface (tablet thickness ca. 1 mm), see figure S1 for higher magnifications. (d) The printed samples are tested mechanically to evaluate performance. The lower panel shows a photograph of a 4-point bending experiment and in the upper panel, the resulting load-displacement curves for five replicate samples of a given design are shown. The tests display near perfect overlap until the yield point, after which, different failure routes are observed.

properties of the system being investigated (Figure 1a), which are then translated into a parametric modeling environment commonly employed in architectural applications^[4] (Figure 1b), which permits the simple modification of key model parameters (e.g., aspects of shape and material) to allow straightforward, highly efficient, and systematic generation of computer models with full 3D complexity and incrementally varying designs (Figure 5 and supplementary video). The designs are then 3D printed to obtain physical, macroscopic objects (Figure 1c) exhibiting a wide range of constituent moduli that, in turn, are mechanically tested (Figure 1d). This pipeline, linking form and function, facilitates highly efficient and high-throughput experimental screening of a very large design parameter space, allowing exploration of the structural design features that play vital roles in a material's performance. The obtained insights serve both as guidelines for the development of new synthetic materials, but also as a method for identification of structural biological concepts inciting further investigation. Here, as an

illustration of efficacy, we apply this framework for studying the structural parameter space of nacre, a robust and damage tolerant biological laminated ceramic composite, lining the interior of many mollusk shells (Figure 2).

Nacre has long been a source of inspiration for the development of new composite materials, through a range of different synthesis strategies.^[3d,5] The intense interest in nacre is due to its intriguing mechanical properties, with the energy to fracture ca. 3000 times greater than that of its main mineral building block, single crystals of aragonite.^[6] The high toughness of nacre has been attributed, in large part, to its “brick-and-mortar” structure, which consists of sheets of individual quasi-hexagonal aragonite tablets, each of which is surrounded by a thin organic matrix (Figure 1a).^[6,7] While its nano- and micro-scale architecture has been extensively studied, the exact roles and relative importance of structural features such as mineral tablet shape and inter-tablet mineral bridges have remained unclear. Using our parametric approach, we constructed a pipeline to allow flexible exploration of several specific features of the structural hierarchy in nacre-like architectures believed to be diagnostic for material performance: the degree of tablet overlap across multiple layers, the regularity of tablet shape within layers, and the degree of material interaction between adjacent layers. Recent advances in multi-material 3D printing allowed us to construct high-resolution nacre structural analogs from a stiff thermoplastic-like polymer to mimic the mineral tablets and a soft elastomer-like phase to emulate the nacre organic matrix. The moduli of the stiff and soft polymers was chosen to differ by more than 1000-fold (the tensile Young's moduli

for the soft and stiff polymer were ca. 0.6 MPa and 2 GPa, respectively),^[8] mimicking the large differences estimated to be found in nature.^[6,9] Replicate nacre-like architectures were also printed in transparent (rigid and flexible) polymers to allow easy visual inspection of fracture behavior and tablet collision during mechanical testing (Figure S2). Three dimensional printed designs were tested in 4-point bending on an electromechanical testing machine (Figure 1d) to emulate one of the challenges nacre can face in nature, for example, as a result of high impact collisions or the crushing predatory behaviors by sea otters, mantis shrimp, or durophagous stingrays.^[10] In Figure 1c, we show a 3D printed nacre-like construct after 4-point bending. The mechanical response of these samples was remarkably reproducible (Figure 1d), with samples displaying near perfect overlap of their mechanical response until the yield point, after which response varied due to the multitude of fracture patterns possible within the composite. Although this our 3D-printing approach does not permit the use of materials

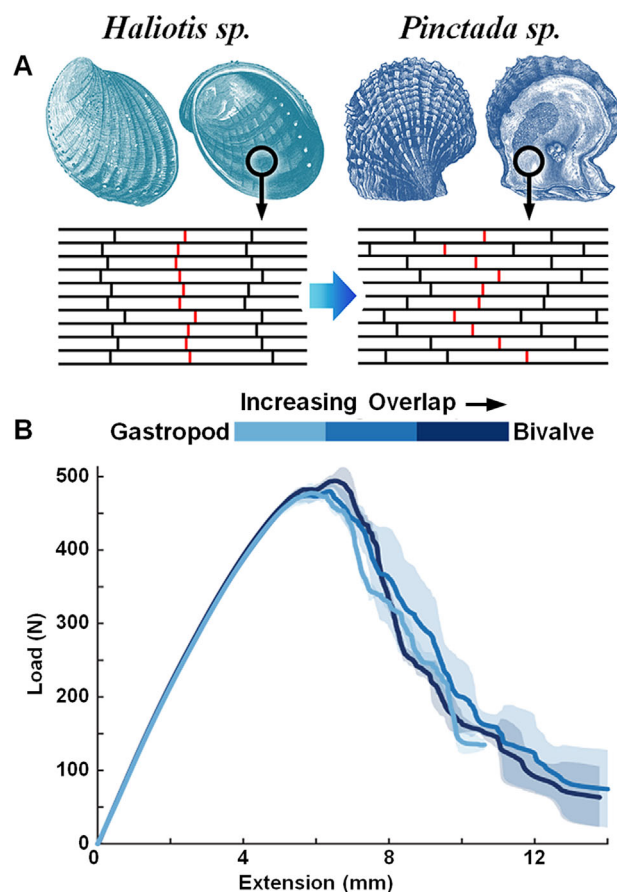


Fig. 2. Comparing the mechanical consequences of percent tablet overlap in bivalve and gastropod nacre. (a) Schematics of transverse cuts through gastropod (e.g., the abalone *Haliotis* sp.) and bivalve (e.g., the pearl oyster *Pinctada* sp.) nacre illustrate the different tablet stacking modes with one end of a selected stack of aragonite tablets highlighted in red. (b) Load-displacement curves for 3D-printed samples with different degrees of tablet overlap. Models were generated to mimic each biological system and investigate an intermediate condition between them: light blue has limited tablet overlap as in gastropods, medium blue represents an intermediate form, and dark blue has significant tablet overlap as in bivalves. The tests are represented by the average of three repeated measurements enveloped by their (shaded) standard deviations.

with the same composition or length scale as those found in the native biological composite, the macroscopic failure mechanisms we observe in the 3D printed nacre analogues are geometrically very similar to those observed in natural nacre,^[11] with cracks forming and propagating through the soft inter-tablet layer in bending and with sliding and pullout of tablets in tension (see details in Figure S2). Because of these qualitatively similar responses to external loading, this approach does permit the direct exploration of specific geometric parameters, whose mechanical contributions can be systematically evaluated and subsequently incorporated into synthetic nacre-like composites.

Since the ability for mollusks to deposit nacre is likely to have evolved independently in gastropods and bivalves,^[12] it is not surprising that gastropod nacre and bivalve nacre are fundamentally structurally disparate, due to the very different stacking modes of their constituent tablets.^[13] The dissimilar stacking originates from the different modes of growth seen in the two classes

of nacre (Figure 2a). In gastropods, nacre grows in a “stack-of-coins”-like fashion, where tablet growth is propagated through mineral bridges connecting adjacent tablets in each vertical stack, while in bivalves, nacre grows in a sheet-like fashion, resulting in a much larger overlap of the tablets from layer to layer, which is more reminiscent of a classical architectural brick-and-mortar geometry. These design differences result in differential interlayer coincidence of the joints between tablets (compare the alignment of red lines in Figure 2a), yet the influence of these different stacking designs with respect to material performance is unknown.

In Figure 2b, the load-displacement curves from mechanical tests of multiple samples of three different nacre-like composites are shown. The tablet overlap varies from gastropod-like to bivalve-like with a transitional step representing a morphological intermediate not found in nature. The curves are almost identical, suggesting that in the evolution of mollusk shell material properties – and for the development of macro-scale synthetic nacre-like composite materials – the degree of tablet overlap is not a parameter that need be carefully optimized. The lack of relationship between tablet overlap and mechanical performance of our tested materials may help to explain the evolutions of nacreous materials that are convergent on a gross structural level (i.e., comprised of stacked aragonite tablets), but strongly disparate in their ultrastructures (i.e., stacking patterns), and yet equally impressive in their mechanical performance.^[13d]

In a second study, we investigated the role of tablet geometry and symmetry within the composite. The shape of the mineral tablets in nacre is quasi-hexagonal,^[14] but in other natural systems, we observe geometries with near-perfect 6-fold symmetry in, for example, honeycombs, snowflakes, and the fascinating guanine crystal arrays governing structural colors in some species of copepods.^[15] In order to understand the influence of tablet shape on material performance, we created models with constant average tablet area, but progressively more random tablet shape. Tile shape was controlled by constructing tilings from arrays of seed points using a Voronoi diagram, where the borders of tiles are drawn along lines equidistant from two seed points. The regularity of the tiling is then dictated by the regularity of the spacing of the seed points: by perturbing a perfect hexagonal array of seed points, the model tilings could be varied from perfectly hexagonal to highly irregular polygons (Figure 3a). As a measure of the irregularity of the structure, we defined the “voronosity”, V , as the standard deviation, σ , of the distribution of angles, α :

$$V = \sigma(\alpha)$$

Testing 3D-printed nacre-like models of varying voronositities (Figure 3a) in 4-point bending, we found that material performance depends dramatically on the regularity of the structural tiling. As the organization of the tablets becomes

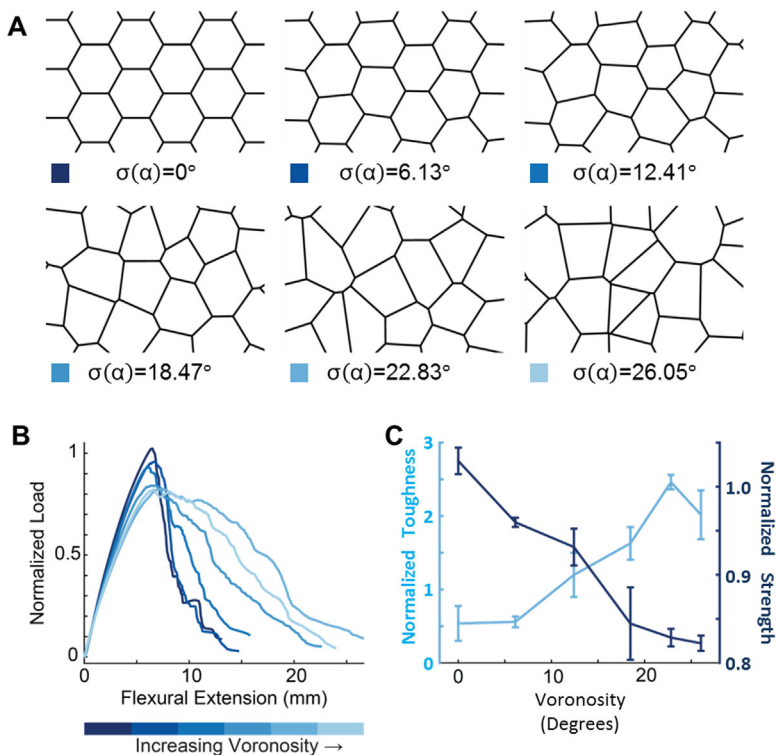


Fig. 3. Influence of tablet shape irregularity on material performance. (a) Schematics from our nacre parametric model showing segments of single tablet layers with progressively more irregular shape, that is, higher voronosity, V (see text for definition). (b) Load-displacement curves for samples with voronosity corresponding to the variations displayed in A. Dark blue represents a regular hexagonal tiling and progressively lighter blue corresponds to more irregular polygonal tilings. All tested samples had the same intermediate tablet overlap. The tests are represented by the average of three repeated measurements enveloped by their standard deviations. (c) Toughness (light blue) and strength (dark blue) calculated for the samples shown in B, showing a performance trade-off between toughness and strength in regards to voronosity.

more irregular, the yield point decreases and the load-displacement curve broadens, meaning that although there is a reduction in load that is required for the onset of failure, it exhibits more post-yield energy dissipation before total failure occurs (Figure 3b). The material performance was further evaluated by calculating the relative strength and toughness (see methods), both of which are shown as a function of voronosity in Figure 3c.

The strength and the toughness of our nacre-like models showed opposite responses with respect to tablet voronosity, with increasing voronosity decreasing the composite's strength, but greatly increasing its toughness. Thus, models with perfectly hexagonal structures can withstand high loads, but then quickly fracture catastrophically, whereas models with high voronosity exhibit lower strength, but dissipate more energy before catastrophic failure occurs. Thus, in the design of a nacre-like tiled composite, there is an inherent conflict between strength and toughness – a key challenge also often faced in engineering applications (see e.g.,^[16]) – and an optimum for maximizing both aspects of materials performance exists in between the perfectly hexagonal and highly irregular tablet shapes. Our results are, therefore,

suggestive of the evolutionary pressures that may have shaped this materials system: natural nacre also exhibits an intermediate voronosity ($V=24.4$), which indicates that tablet shape could have been optimized for shell mechanical properties that balance load resistance and energy dissipation to provide protection for the organism. Interestingly, the optimum voronosity in our 3D printed nacre differs slightly from that of its biological analogue, reflecting differences in the mechanical properties of the two systems and suggesting that evolution may place a premium on toughness over absolute strength.

Many different toughening features have been proposed to account for the impressive mechanical properties of nacre, such as nanograin rotation and deformation, tablet waviness, and mineral bridges.^[2a,7a,9,17] As a last feature, we investigated the latter by testing the performance effects of introducing mineral bridges into our nacre-like composite. The presence of mineral bridges, peg-like asperities spanning the organic layer between tablets, in nacre has been known for many years,^[18] but their exact role continues to be debated: Are the bridges a consequence of how the biomineral grows or do they have an important functional role in mechanical performance?^[7a,18,19] Experimentally evaluating the mechanical role of the mineral bridges is not directly feasible, as no biological bridge-free control is available for tablets of a defined overlap (for example, there are no known examples of gastropod nacre lacking mineral bridges), so instead, we investigated the effect of these stiff, vertical tablet-spanning bridges in our nacre-like composite.

Three models with different interlayer mineral bridge density were evaluated and all models exhibited intermediate tablet overlap and voronosity (Figure 4a). The mineral bridges were modeled with a square cross-sectional geometry, in order to minimize the computational demands during the generation of STL files for 3D printing. Because of their small size and the inherent resolution limits of the fabrication platform used in this study, the resulting 3D printed mineral bridges were approximately circular in cross-section (Figure S3), similar to what is seen in biological nacre. Increasing the density of bridges in our 3D printed nacre mimics, resulted in a substantial reduction in the performance of the material in 4-point bending (Figure 4b), with the maximum sustainable load in the high bridge-density model only $\approx 75\%$ of that of the model lacking bridges. One possible hypothesis for this, perhaps surprising, trend is that the bridges act as stress transmitters, ferrying the load from one tablet layer to the next, partially bypassing the energy dissipating effects of the organic interlayers, and consequentially reducing the composite's global performance.

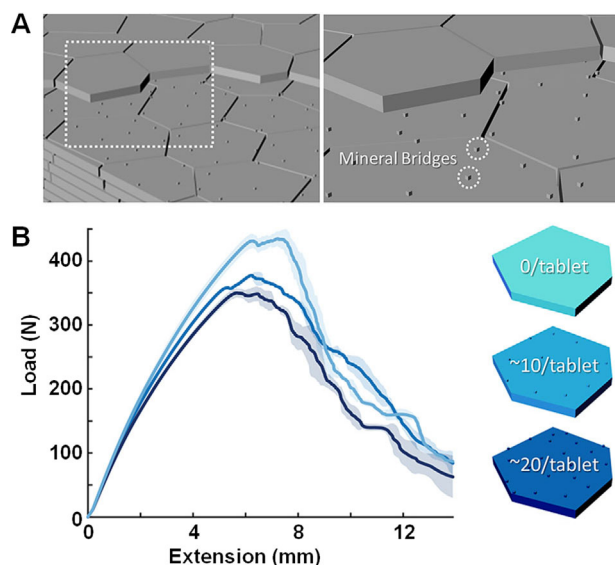


Fig. 4. (a) 3D rendered models with mineral bridges showing (for illustrative purposes) only the stiff components of the nacre-like composites. (b) Load-displacement curves for 3D printed models with increasing bridge density, illustrating a negative effect of stiff interlayer bridges on strength. Light blue is a control sample without bridges. Medium blue is the same sample with ca. 10 bridges per tablet and the dark blue case is with ca. 20 bridges per tablet. The tests are represented by the average of three repeated measurements enveloped by their standard deviation.

The results from the studies described here provide valuable information for the rational design of new nacre-like composites, where the addition of support between layers would likely not be favorable. We note, however, that at the sub-micron scale, the mechanical properties of materials depend strongly on feature size,^[20] meaning that nanostructured materials may have a dramatically different toughness than their macroscopic analogs. Although our 3D printed

models preserved the relative size ratios of their structural features, the 3D printing technology implemented in this study prevented us from fabricating our nacre-like composites at biologically relevant length scales. As a result, even the smallest structures, the mineral bridges, were macroscopic. While, at the length scale of natural nacre, there may be other mechanical consequences of the mineral bridges, the results shown here clearly demonstrate that a detailed feature-level understanding of structure-function relationships in complex biological composites is required before the cost-effective and rational design of macro-scale structural analogs can be considered.

Our parametric modeling approach offers significant benefits over conventional methods and can provide important insights into the design and function of structural biological materials. While 3D printing has been applied previously to investigate the performance of biological materials, the resulting tangible models were either direct mimics,^[10a,21] or highly simplified structural analogs of the biological structures under study.^[3a–c,22] The strength of the parametric workflow presented here is that it allows for the flexible and high-throughput creation of true 3D models (Figure 5 and supplementary video) and can be applied to any system with features amenable to modeling and 3D printing. In particular, the workflow can be easily implemented in those systems, where material geometries and properties are already known to some degree. Furthermore, it allows the construction of rich, “high resolution” structural parameter spaces around these baseline anatomies, not only from readily available specimens, but also from those that are rare, pickled in museums, extinct, or have never existed in nature, allowing inquiry

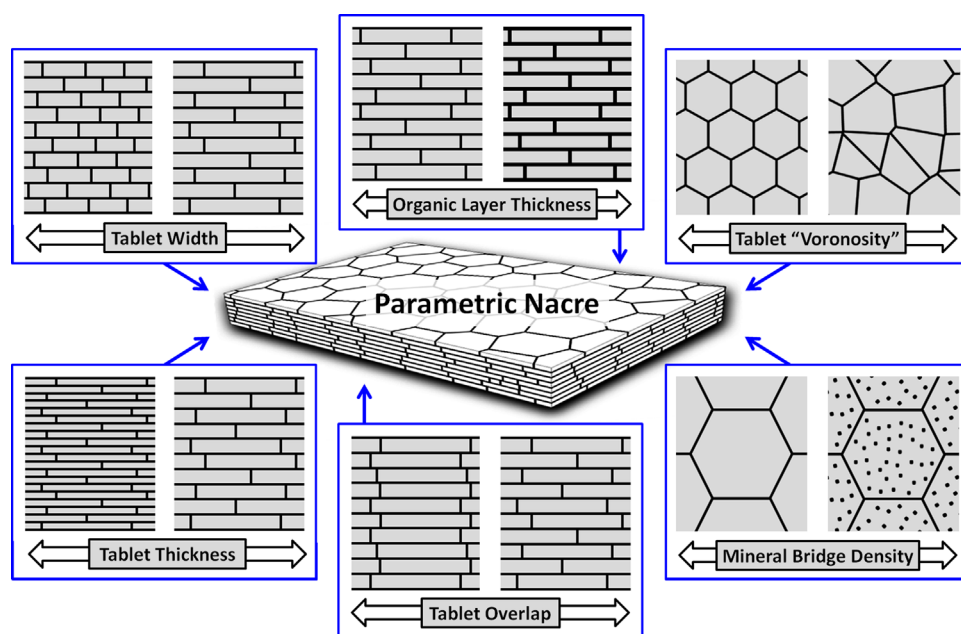


Fig. 5. Conceptual schematic of the parametric work flow for the generation of 3D printable nacre mimics (see also Fig. S4 and supplementary video). In this example, six different nacre parameters are diagramed, each of which can be individually varied through manually moving the sliders (denoted by white arrows) within the 3D modeling environment.

into the form-function relationships that shape biodiversity. This interdisciplinary approach opens myriads of opportunities for knowledge exchange among the biological, engineering, and materials sciences, strengthening understanding across disciplines, and creating avenues for the fabrication of biologically inspired materials tailored for their specific applications. While the length scales and the material selections probed using this framework are dictated by the capabilities of the 3D printing technique, 3D printing technologies are increasingly available outside of industry and are developing rapidly, allowing for even more material types to be printed and with a better resolution.^[22a,b,23] These developments will allow for studies, where not only "geometric" structural parameter spaces can be probed, but also where design principles can be effectively tested at length scales and using materials directly relevant to biology, thus strongly facilitating future material design and structural optimization.

1. Methods

Parametric modeling, meshing and computer-aided design (CAD) rendering were carried out using Grasshopper for Rhinoceros 5 (ver. 0.9.0076, Robert McNeel & Associates, WA, US), a conceptual schematic of which is shown in Figure 5 (with technical details in Figure S4). The parametric nacre model created in Grasshopper was designed to allow for the straightforward creation of models that included the main features of interest in nacre. As such, the model includes sliders that permit rapid and straightforward modification to the overall size of the structure, dimensions of the two in-plane directions, number of layers, tablet size, tablet thickness, layer spacing (organic interface thickness), tablet shape, tablet overlap, density of mineral bridges, and density of nano-asperities. Each feature can be changed independently and all random events (e.g., perturbation of platelet shape) can be instantly regenerated, in order to create different models with the same "degree of randomness" (see supplementary video). The model was also designed to provide readouts (Model Parameter Summary) for monitoring the different model characteristics. For example, the volume ratio of the soft polymer varied by less than 0.5%, when varying the voronosity from the lowest to the highest value and when introducing mineral bridges.

The parametric 3D models obtained by the program in Figure S3 were printed on a multi-material Connex500 3D printer (Stratasys, Rehovot, Israel). For the stiff phase of the nacre-like composites, either VeroWhite (RGD835) or VeroClear (RGD810) were used (Stratasys, Rehovot, Israel). For the soft polymer phase, the transparent TangoPlus (FLX930) was used (Stratasys, Rehovot, Israel). After printing, the samples were left to rest for at least 24 h before testing to ensure complete curing of the material. Since the material properties of the polymers can change as a function of aging, samples in the same testing series were printed and tested together. For larger test series, however, it was not possible to print all samples simultaneously due to the limited build area

of the 3D printer. In these cases, standard (control) samples with known mechanical responses were printed in each print job and tested together with the other samples. The load response of the tested samples was then normalized to that of the corresponding standard.

Electromechanical testing was performed with a 10 kN load cell on an Instron 5566 using a constant load rate of 2 mm min⁻¹ (Instron, Buckinghamshire, UK). The dimensions of the samples were adapted to match the 4-point bend fixture with a 1:2 ratio between the separation of the loading and support pins, see Figure S1.

Scanning electron microscopy analysis of the fracture surface post-failure and natural nacre was performed with a Tescan Vega III GMU SEM (Tescan, Brno, Czech Republic). For imaging of natural nacre, the shell of an adult (19 cm) red abalone (*Haliotis rufescens*) was fractured into 1 cm fragments and then cleaned with a 5.25% solution of sodium hypochlorite for 6 h. The resulting shell fragments were fractured longitudinally to create planar surfaces, which were mounted to aluminum pin mounts with conductive carbon tape and imaged by SEM. The voronosity was calculated from the acquired images by segmenting out the inter-tablet layer using a machine learning algorithm.^[24] The resulting image was then skeletonized^[25] and the angles of the obtained lines semi-automatically determined using MATLAB. In total 676 angles were measured resulting in a calculated average voronosity of $V = 24.4^\circ$.

To allow better optical visualization of the clear 3D printed composites, for example, for photographic purposes (Figure 1c, S2D), the samples were immersed in PEG-400 (Sigma-Aldrich), which masked some of their fine scale surface roughness.

For the extraction of material properties of the models with varying voronosity, the strength was defined as the maximum load carried by the material:

$$\text{strength} = \sigma_{\max}$$

To illustrate the different in material behavior post-yield the toughness was found by integrating the stress-strain curve from $\sigma_{\max}$ to 30% of $\sigma_{\max}$:

$$\text{toughness} = \int_{\sigma_{\max}}^{0.3\sigma_{\max}} \sigma \, d\epsilon$$

Alternatively, another measure of toughness can be calculated by integrating the full stress-strain from zero to the largest extension reached by all samples. The result of this calculation is shown in Figure S5.

2. Author Contributions

HB, JCW, and SF designed the research. SF conducted the parametric modeling. SF and JCW performed the

experiments. HB, JCW and SF analyzed the results. SF, MND, HB, and JCW wrote and edited the manuscript.

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